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TRANSACTIONS

Oxidation of Tea Tannin by the Action of Oxidizing Enzymes in Fresh Tea-Leaf.

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(Agricultural Chemical Department, Taihoku Imperial University, Taiwan.)

Received May 27, 1940.

In Taiwan, the manufacturing of black tea has become prosperous year after year and its progress is very remarkable. However, we must acknowledge the superiority of the tea made in India and Ceylon to that which is made in Taiwan. The quality of black tea is due to its colour, taste and flavour in the liquors. In the process of manufacturing, that is, in the operation of withering, rolling and fermentation, tannin is oxidized by enzyme and it is said that the unique colour, flavour and taste are thus produced. We intend to contribute to the improvement of the quality of black tea from both sides by studying tannin and enzyme, which are the chief ingredients of raw tea-leaves.

About the tannin of tea leaves, several reports(1) have been published up to the present time. One of the writers, Osima(2) has made a report about the tannin of Taiwan raw tea leaves. There are the reports by Mann and Aso about the enzyme of tea leaves, while about the oxidizing enzyme of Taiwan raw tea leaves Hayasi(3) has made a report. While recently very interesting results are reported by Lamb and Roberts.(4)

Now when we make the enzyme solution of tea leaves act on the pure colourless tannin, the tannin becomes reddish brown, like the infusion of black tea. Thus we can infer that the colour of black tea is produced by the change of tannin by enzyme. Moreover, by separating the substance produced by the change of tannin we can ascertain that it is an oxidated product. We have just taken the first step in this study and report the results which have been obtained so far.

THE PROPERTIES OF OXIDIZING ENZYMES.

The tannin and enzyme which we use are as follows.

Tannin Solution: 1% aqueous solution of a crystal of colourless gallocatechin separated from raw leaves(2).

Enzyme Solution: Grind thoroughly the fresh young leaves in the mortar. Add to it water of twice its quantity and a small quantity of toluol. Make extraction by shaking for two hours. Leave it one night in a cool place and filter by a centrifugal separation. Adding aluminium oxide hydrate, pure, free from alkali ($Al_2(OH)_6$) to the fluid and again by filtrating, tannin and other impurities are removed and we get a light yellowish clear solution. This is the enzyme solution. The nature of the oxidizing enzyme contained in this solution may be explained as follows.

A. Peroxidase.

(a) The optimum hydrogen ion concentration.

Put 1 cc., 0.1% guajacol solution, 1 cc., 0.1% hydrogen peroxide, 1 cc Mc Ilvaine's standard buffer solution and 4 cc distilled water in a test tube. Added to it 2 cc enzyme solution and made it act on the solution in the tube at the temperature of 35°C for 15 minutes. And studied the colour-tone of tetraguajacol thus produced, measuring by Rosenheim-Schüster No. 91 tintometer on Lovibond's colour system. As a control experiment, we used enzyme solution boiled for five minutes. The result obtained is as follows.

Table I.

pH	Red value	pH	Red value	pH	Red value	pH	Red value
3.0	7.2	3.8	15.0	4.6	15.5	5.4	13.0
3.2	9.1	4.0	16.1	4.8	14.9	5.6	11.9
3.4	13.1	4.2	16.3	5.0	14.2	5.8	10.9
3.6	14.7	4.4	16.5	5.2	14.0	6.0	10.1

So far as this experiment goes the optimum hydrogen ion concentration of peroxidase-action is at pH 4.4.

Moreover we ascertained that within the scope of this experiment, peroxidase action reached its maximum when 1 cc., 0.1% hydrogen peroxide solution was used proportionally with 1∼2 cc enzyme solution.

(b) The optimum temperature.

Using 1 cc enzyme solution and 2 cc buffer solution (pH 4.4) and in accordance with the above mentioned experiment (a), measured the optimum temperature of peroxidase action.

Table II.

Temperature	25°C	30°C	35°C	40°C	45°C	50°C
Red value	6.9	7.8	9.1	10.8	10.8	11.2
Temperature	55°C	60°C	65°C	70°C	75°C	80°C
Red value	11.3	10.7	9.3	4.9	0.9	0.1

The optimum temperature is 50∼55°C.

It is shown by another experiment that peroxidase action is hindered by tannin. In case of measuring peroxidase action, if the quantity of tannin contained in the whole quantity of 10 cc was less than 0 05 mg., there was no hindrance in the colouring reaction and peroxidase could not be adsorbed by aluminium oxide hydrate, and so peroxidase action can be perceived when tannin is removed from enzyme solution containing tannin. As for the causes of these, we must look for their explanation after future study.

B. Oxidase.

(a) The optimum hydrogen ion concentration.

Measured the optimum hydrogen ion concentration in oxidase action by indophenol reaction. Put 1 cc indophenol reagent (the mixture of 1 cc 1% *p*-phenylendiamin solution, 1 cc 1% *α*-naphthol solution dissolved in 50% alcohol, 2 cc 90% alcohol, 6 cc distilled water. Each must be mixed just before using.), 2 cc buffer solution, 6 cc distilled water, 1 cc enzyme solution in a test tube and mixed them. Then measured the reddish purple colour which was produced after thirty minutes at the temperature of 35°C. As a control test, used enzyme solution boiled for 5 minutes.

Table III.

pH(*)	Red value	pH(*)	Red value	pH(**)	Red value
3.0	0.3	7.2	8.5	8.0	10.2
4.0	0.8	7.4	8.8	8.3	10.0
5.0	2.0	7.6	9.2	8.5	9.7
6.0	5.0	7.8	9.8	8.7	9.2
7.0	8.0	8.0	10.2		

(*) McIlvaine standard buffer.
(**) Sørensen's borate buffer.

In this study, the optimum hydrogen ion concentration in oxidase action was near pH 8.0.

(b) The optimum temperature.

Using 1 cc enzyme solution and 2 cc buffer solution (pH 8.0), measured the optimum temperature in oxidase action under the above mentioned condition (a).

Table IV.

Temperature	25°C	30°C	35°C	40°C	45°C	50°C	55°C
Red value	7.8	8.7	9.8	11.0	11.6	12.6	12.2

Temperature	60°C	65°C	70°C	75°C	80°C	85°C
Red value	12.2	10 9	8.7	5.0	3.8	2.2

The optimum temperature is 50～65°C.

Oxidase action was not affected by tannin at all and oxidase was not adsorbed to an aluminium oxide hydrate.

THE CHANGE OF CATECHINES EFFECTED BY ENZYME SOLUTION.

We put 2 cc tannin solution, 2 cc enzyme solution, and 1 cc McIlvaine standard buffer solution in a Thunberg tube. After the air in the tube was replaced by oxygen or nitrogen we mixed these solutions. When we had kept the mixture for 20 hours at the temperatures of 35°C, we observed the change of colour. Under the influence of oxygen, the solution gradually became yellowish red or reddish brown. However, under the influence of nitrogen the colour of the solution scarcely changed. On the contrary, the enzyme solution when it was boiled, produced almost no change in nuance in every case. We measured the colour-tones of the solutions which thus changed their colours, by using the tintometer of Rosenheim-Schüster No. 91 on the Lovibond colour system. The result was as follows.

Table V.

pH	Enzyme solution	Gas in the reaction tube	Degree of colouration	
			Yellow	Red
5		Oxygen	4.1	19.1
		Nitrogen	1.0	4.0
	boiling	Oxygen	1.1	3.9
	boiling	Nitrogen	0.8	1.0
6		Oxygen	8.0	29.0
		Nitrogen	1.8	5.0
	boiling	Oxygen	1.8	8.0
	boiling	Nitrogen	0.7	1.3
7		Oxygen	23.3	19.5
		Nitrogen	8.0	14.2
	boiling	Oxygen	9.1	16.0
	boiling	Nitrogen	2.3	2.5

The degree of the change of colour differs according to pH and the colour becomes more intense and the colour-tone differs as pH nears neutrality. In acid case, under the influence of nitrogen gas, there is scarcely any change of colour, and in the case of boiled enzyme solutions, under the influence of oxygen, there is almost no change of colour, either. In the case of pH there is more or less colouring in every case, but compared with the case where the enzyme is made to act, the degree of colouring is very slight.

Still, for the sake of comparison, we observed the reaction in the same way, using *d*-catechin, tea-tannin and synthetic bisflavpinacol(2).

Although we cannot see what kind of chemical change happened, it is clear

Table VI.

pH	Gas in the reaction tube	Tannin solution	Enzyme solution	Degree of colouration	
				Yellow	Red
6	Oxygen	*d*-catechin		10.2	30.0
			boiling	1.0	4.1
		tea tannin		14.8	37.3
			boiling	2.6	4.5
		bisflavpinacol		22.2	38.3
			boiling	3.0	6.3

from the result above that the enzyme solution which we use here, exerts action on not only catechin but also on tannin and has the action to change them into reddish brown substances.

Accordingly we know that tannin is changed by the enzyme action and is coloured as the result, and also that oxygen is necessary in this case. In the process of manufacturing black tea, such a change happens naturally and we can say that the colour of black tea is thus produced.

OXIDATED PRODUCT OF CATECHINS EFFECTED BY THE ENZYME.

In order to get the coloured-substance through the above experiment, we made an experiment under the proper condition and we got the precipitation of coloured substance from the same reaction in both cases of *d*-catechin and gallocatechin.

Oxidation of *d*-catechin: dissolved 1 g. *d*-catechin in 30 cc water, and added to it 30 cc enzyme solution and 10 cc buffer solution (pH 6.0). Letting in oxygen, stirred it. Left it for 24 hours at the temperature of 40°. Then the mixed solution gradually changed from yellowish red to red. And finally yellowish red precipitation was produced. Next separated the precipitation by filtration and washed with water and dried it. And then we got reddish brown powder which was insoluble in water and was difficult to dissolve in alcohol.

Oxidation of gallocatechin: dissolved 2 cc gallocatechin in 20 cc water. Added 50 cc enzyme solution and 10 cc buffer solution (pH 6.0), and let in oxygen, and made it act on the mixed solution for about 60 hours at the temperature of 40°. And then the solution gradually changed from yellowish red to red and finally reddish brown precipitation was produced. Separated the precipitates by filtration and washed with water and dried it. Then the reddish brown powder, which was insoluble in water and difficult to dissolve in alcohol, was produced.

The elementary composition of these substances is as follows.

	C(%)	H(%)	O(%)	
d-catechin $C_{15}H_{14}O_7$	62.07	4.83	33.10	
Oxidated product of *d*-catechin	50.07~51.44	5.04~5.30	44.07	(average)
Gallocatechin $C_{15}H_{14}O_7$	57.81	4.61	37.58	
Oxidated product of gallocatechin	50.11~51.78	4.76~4.90	44.22	(average)

The coloured substance produced by enzyme action shows a remarkable decrease of carbon and a remarkable increase of oxygen. Therefore, we consider the change as a kind of oxidation.

We get the reddish brown acetyl compounds by acetylating the oxidated products above mentioned with pyridine and anhydrous acetic acid in both cases. If we compare the acetyl compound of catechin with the acetyl compound of oxidated product from catechin, as can be seen by the following chart, there is a little quantity of acetyl radicals in the latter and then a decrease of free hydroxyl radicals. And this fact is a notable change.

	CH_3O(%)
Acetyl compound of *d*-catechin $C_{15}H_9O(O \cdot CO \cdot CH_3)_5$	43.00
Acetyl compound of oxidated *d* catechin	15.06~15.17
Acetyl compound of gallocatechin $C_{15}H_8O(O \cdot CO \cdot CH_3)_6$	46.23
Acetyl compound of oxidated gallocatechin	36.34~36.45

When tannin is oxidized by enzyme, probably after various steps, it becomes an insoluble substance, that is to say, phlobaphene. In the course of the steps a soluble oxidized product is obtained, and that is how the colour of black tea is produced.

(1) Deuss: Rec. Trav. chim., **42**, 496, (1923).
Shaw: Thea Tannin (1932).
Tujimura: Sci. Paper Institute Physical and Chem. Research., **14**, 261, (1930); **26**, 186, (1935).
(2) Osima: J. Agr. Chem. Soc. Japan, **12**, **1**, (1936).
(3) Hayasi: J. Soc. Trop. Agr. Taiwan, Japan, **6**, 388, (1934).
(4) Lamb and Roberts: Nature **18**, 867, (1939).

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

Researches on Bamboos in Taiwan as a Raw Material for Pulp. Part III.

(pp. 621∼625)

By Minoru TUTIYA and Setuo FUKUHARA.

(Industrial Research Institute of Taityu, Taiwan; Received June 4, 1940.)

Studies on the Vegetable Tannins in Taiwan. Part 6.

Manufacture of Tanning Extract from the Bark of *Acacia confusa*. II.

(pp. 626∼630)

By Yasuyosi OSIMA, Zensaburo SIRAKI and Zenyu HYO.

(Agricultural Chemical Department, Taihoku Imperial University, Taiwan;
Received June 8, 1940.)

As the result of studying the extraction of bark with alkali or acid solution, we found that the maximum yield of tannin could be obtained with 0.1% HCl, H_2SO_4 or SO_2 solution. We made tannin extract with 0.1% SO_2 solution, which we thought most practical, and examining the chemical properties, diffusion velocity into gelatin-gel, and absorption amount of tannin by hide powder, we got good results in all cases.

Chemical Researches on the Pulp Woods of Manchoukuo. Part VI.

Fibre-length, Chemical Analysis and Cooking Experiment of the Hard Wood.

(pp. 631∼640)

By Masuzo SHIKATA and Yoshitsugu KIMURA.

(Kyoto Imperial University; Received June 10, 1940.)

In this paper, the researches on the chemical components, fibre-length, and cooking experiments of hard woods are given.

The species of the woods employed are as follows;

Japanese name	Scientific name	Annual rings
Ominonire	*Ulmns Macrocarpa.* Hance.	107
Ohyonire	*UlmusLaciniata.* Mayr.	71

1. Physical properties.

The distribution of fibre-lengths is as shown in Table 1.

Table 1. The Distributions of Fibre-length. (Interval 0.1 mm).

Length (mm)	Ominonire	Ohyonire	Length (mm)	Ominonire	Ohyonire
0.3~0.4	4.0	0.6	1.5~1.6	0.4	3.6
0.4~0.5	5.4	1.6	1.6~1.7	0	3.6
0.5~0.6	5.8	2.6	1.7~1.8	0	2.8
0.6~0.7	8.0	3.0	1.8~1.9	0.2	0.4
0.7~0.8	8.8	3.4	1.9~2.0	0	0.8
0.8~0.9	19.2	5.2	2.0~2.1	0	0.4
0.9~1.0	15.6	9.6	2.1~2.2	0	0.6
1.0~1.1	15.4	9.2	2.2~2.3	0	0
1.1~1.2	11.2	18.0	2.3~2.4	0	0
1.2~1.3	7.2	15.2	2.4~2.5	0	0
1.3~1.4	2.4	13.0	2.5~2.6	0	0.4
1.4~1.5	1.6	6.0			

The data given in Table 1 are graphically shown in Figure 1. Those data show that the fibre-lengths of these hard woods are not so long as those of soft woods, but are nearly equal to those of other hard woods.

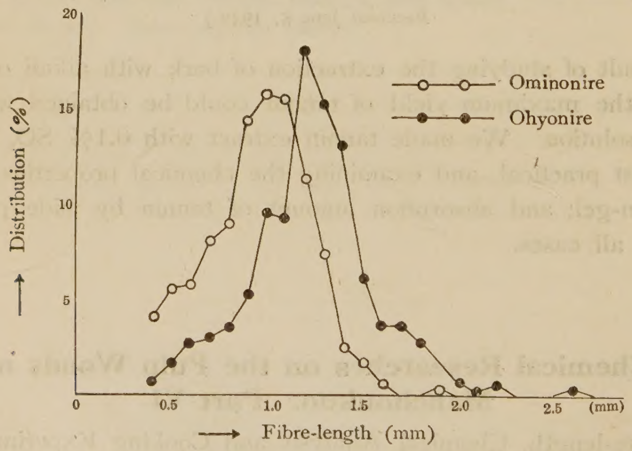

Fig. 1.

2. Chemical analysis of wood components.

The results of analysis of the chemical components of these woods are given in Table 2.

Table 2. Chemical Components (% Oven dry).

Species / Components	Ominonire	Ohyonire	Species / Components	Ominonire	Ohyonire
Alcohol-benzene-soluble	1.97	1.48	Hemi-cellulose	22.01	23.10
Water-soluble	0.87	1.12	Nitrogen	0.12	0.15
Hot-water-soluble	1.59	1.65	Crude protein	0.76	0.95
1% NaOH-soluble	13.16	16.76	Ash	1.03	0.83
Crude cellulose	54.85	56.22	Methoxyl	6.92	7.30
α-cellulose	36.61	39.96	Methoxyl/Lignin × 100	30.70	32.42
β-cellulose	9.22	10.21	Ca-pectic acid	2.31	2.77
γ-cellulose	9.02	6.05			
Lignin	22.54	22.52	In Total cellulose α-cellulose	66.75	71.08
Pentosan	21.90	23.01	β cellulose	16.81	18.16
Mannan	0.00	0.00	γ-cellulose	16.44	10.76
Galactan	0.11	0.09	Volume weight	0.49	0.55

3. Cooking experiments.

The cooking experiments with the Ca-sulphite process and sulphate process were carried out under the conditions given in Table 3.

Table 3. The Conditions of Cooking Experiments.

Run Number	A—1	A—2	A—3	B—1	B—2	C—1
Method / Condition	Ca-Sulphite Process					Craft Process
Cooking solution	CaO 1(%) Total SO_2 7(%) Free SO_2 5.8(%)	〃 〃 〃	〃 〃 〃	〃 〃 〃	〃 〃 〃	NaOH 7.5 (%) Na_2S 1.75(%) Na_2CO_3 1.75(%)
Chip/ cooking solution	20 g/100 cc	14.3 g/100 cc	〃	〃	〃	〃
Penetration	110°C, 4 at. p.* 2 hrs.	〃 〃	〃 〃	〃 〃	〃 〃	〃 2 at. p. 〃
Main cooking	130°C, 6 at. p.** 3 hrs.	〃 5	〃 7	135°C 〃 5	〃 7	160°C 〃 2
Total cooking time	8.5 hrs.	10.25	12.75	11.0	13.0	7.25

* at. p: Atmospheric pressure.

** 6 at. p: Maintain 6 at. p. by blowing.

The chemical components of unbleached pulps are given in Table 4.

Table 4. Cooking Data.
The Analysis of Unbleached Pulps and Yield.

Wood	Components \ Run Number	A—1	A—2	A—3	B—1	B—2	C—1
Ominonire	Ash		0.59	0.79	0.92	0.65	2.29
	α-cellulose		80.42	83.58	78.88	84.83	28.26
	β-cellulose		7.33	6.81	11.95	5.66	8.62
	Pentosan		5.01	3.87	5.82	4.59	13.24
	Copper number		3.82	3 20	2.72	2.62	0.62
	Roe's number		6.07	4.82	4.51	3.16	3.02
	Yield to chip		45.84	47.37	39.37	37.5	38.65
	Yield to 1 m³ wood (kg)		225	232	192	184	189
Ohyonire	Ash	2.11	0.55	0.40	0.72	0.62	2.56
	α-cellulose	71.91	82.26	84 71	82.45	84.69	85.54
	β-cellulose	12.48	7.71	6.95	10.48	4.64	10.09
	Pentosan	7.26	5.01	2.77	4.93	6.17	10.35
	Copper number	2.57	2.26	2.97	2.60	2.65	0.76
	Roe's number	12.79	4.64	3.03	3.04	2.86	2.17
	Yield to chip	52.47	46.35	47.89	42.75	41.70	39.54
	Yield to 1 m³ wood (kg)	289	255	263	235	229	217

4. Bleached pulps.

The unbleached sulphite- and sulphate-pulps of each wood were bleached by the two stages method.

After applying chlorine gas amounting to about 75% of theoretical bleach requirement, which was calculated by "Roe-number," they were washed with about 0.03% NaOH and water, and steeped in bleaching powder solution of about 0.18% for 0.5 hours to 7 hours at room-temperature.

The chemical components of each bleached pulp are shown in Table 5.

Table 5. Bleached Pulps.
The Analysis of Bleached Pulps and Yields.

Wood	Components \ Run Number	A—1	A—2	A—3	B—1	B—2	C-1
Ominonire	Ash		0.28	0.31	0.28	0.28	0.44
	α-cellulose		81.84	86.79	82.01	88.84	89.05
	β-cellulose		17.01	9.49	10.70	6.50	8.87
	γ-cellulose		1.15	3.62	7.29	4.66	2.08
	Pentosan		4 32	3.41	5.00	3.75	10.35
	Copper number		2.55	1.06	1.14	1.10	0.54
	Yield to chip		37.48	39.47	34.34	33.14	29.07
	Yield to 1 m³ wood (kg)		184	194	168	162	151

Ohyonire	Ash	1.00	0.33	0.18	0.30	0.38	0.59
	α-cellulose	82.94	84.06	88.38	87.84	87.59	89.84
	β-cellulose	8.54	11.86	6.19	10.54	7.16	9.61
	γ-cellulose	8.52	4.08	5.42	1.62	4.25	0.55
	Pentosan	6.13	4.43	2.68	4.08	2.38	9.07
	Copper number	4.47	0.95	1.41	0.65	0.73	0.61
	Yield to chip	38.41	39.89	42.11	37.57	36.64	34.73
	Yield to 1 m³ wood (kg)	211	218	231	207	202	191

Conclusion.

The sulphite pulps showed lower pentosan content and lower ash content.

In general, Ohyonire were superior to Ominonire with regard to the pulp yields and to the α-cellulose contents, pentosan.

It seemed that with respect to the sulphite process, low temperature and long time cooking is comparatively good.

Biochemical Studies on Glutathione. Report XIII.

Relation between the Administration of Diet and the Glutathione Content of Arterial and Venous Bloods.

(pp. 641~648)

By Masayoshi Ogawa.

(Department of Nutrition, College of Medicine, Nippon University;
Received June 3, 1940.)

In the previous communication the author reported some correlations between the glutathione contents of arterial and venous blood of normal rabbit (glutathione was determined by the method of Okuda and Ogawa.).

In the present report he investigated the effect of administration of diet upon the glutathione (GSH, GS–SG) content of arterial and of venous bloods, employing several normal rabbits.

These animals were phletomized at intervals before and after feeding. The results obtained are shown in the following table.

Glutathione Content of Blood before and after Meal Feeding.

Hours observed after meal.	GSH		GS-SG		Total	
	Arterial	Venous	Arterial	Venous	Arterial	Venous
before meal	100 (90.1)	100 (100)	100 (132.3)	100 (100)	100 (100.5)	100 (100)
1/2 hours after meal	93.0 (88.2)	97.0 (100)	167.9 (189.9)	104.5 (100)	123.1 (124.8)	100 (100)

1 hours after meal	97.0 (89.1)	98.3 (100)	145.3 (169.6)	105.9 (100)	114.9 (114.5)	100.5 (100)
2 hours after meal	102.3 (93.1)	101.1 (100)	150.7 (164.9)	108.0 (100)	121.7 (118.8)	103.9 (100)
4 hours after meal	103.1 (81.4)	104.9 (100)	143.9 (190.2)	113.3 (100)	119.0 (111.2)	107.1 (100)
6 hours after meal	99.4 (93.4)	95.8 (100)	145.4 (171.4)	108.0 (100)	115.0 (116.1)	99.1 (100)
8 hours after meal	93.8 (86.1)	104.3 (100)	151.5 (172.7)	97.5 (100)	111.4 (108.7)	102.4 (100)
10 hours after meal	100.3 (87.1)	100.6 (100)	107.3 (145.3)	92.6 (100)	103.6 (105.2)	98.0 (100)
12 hours after meal	101.7 (89.3)	105.1 (100)	98.5 (132.8)	87.8 (100)	100.4 (101.4)	99.6 (100)

As shown in the above table, in every case, the GSH contents of arterial blood is less than that of venous blood, while the GS–SG contents of arterial blood is greater than that of venous blood. The total glutathione contents are the same in the case of fasting.

It is a very interesting fact that GS–SG content of arterial blood is conspicuously increased immediately after feeding and within 10～12 hours it retuns to normal condition, while GSH and GS–SG content of venous blood are not conspicuously influenced throughout the experiment.

Studies on Ascorbic Acid (III).

On the Action of Ascorbic Acid on Glutathione. (I).

(pp. 649～652)

By Kichinosuke Fujimura.

(The Institute of Chemical Rerearch, Kyoto Imperial University;
Received June 12, 1940.)

Exchangeable Calcium and Magnesium of Soils in Tyōsen. (I.)

(pp. 653～662)

By Misu-Hideo.

(Agricultural Experiment Station, Government General of Tyōsen;
Received June 6, 1940.)

On the Metabolism of Organic Acids by Bacteria. I~II.

(pp. 663~686)

By S. TADA.

(Agricultural Chemical Laboratory, Tokyo Imperial University;
Received June 12, 1940.)

Studies on the Value of Chemicals as Manure for *Juncus effusus* L. var. *decipiens* BUCH.

Report II.

On the Effect of Different Kinds of Potash Salts.

(pp. 687~695)

By H. SUTOH.

(The Prefectural School of Agriculture and Fosestry, Masuda, Simane, Japan;
Received June 8, 1940.)

A report has already been made by the author on the relative value of different kinds of nitrogen compounds. In this paper the result of experiments carried on with different kinds of potash salts will be presented. The potassium salts used in the present experiment were potassium carbonate, potassium sulphate, potassium chloride, potassium bromide, potassium iodide, potassium bichromate, potassium chlorate and potassium permanganate as potassium source. Several lots of these salts, of no potassium salt, and of no manure, were set up in a greenhouse. The conditions of growth, yield, together with the quality of the rush were investigated.

The results obtained are summarized as follows:

(1) The rush in the lots of K_2CO_3, K_2SO_4, KCl, KBr and $KMnO_4$ were all observed to cause the normal growth in length of the stem till late in October and then slackened. In winter season, the top of the rush began to wilt and the wilting gradually extended down toward the root as far as 2/5 part of the stem. This wilting was especially remarkable in the lots of KBr and $KMnO_4$.

(2) The tillering continued as late as the harvesting time in the lots of K_2SO_4, $KMnO_4$, KCl, K_2CO_3, and also those with no potassium, tillers increasing in number ranging from about 18 in no potassium lots to about 26 times the original number in the K_2SO_4-lots.

(3) The comparative yield of air-dried stems was 135 in the lot of K_2SO_4, 121 in the KCl-lot, 119 in the K_2CO_3-lot, 117 in the $KMnO_4$-lot, 114 in the KBr-lot, and 100 in no potassium lot.

(4) Under the condition in which this experiment was carried out KCl was useful in increasing the yield of long stem, although the total yield in this case was less than that in K_2SO_4 lot, as against the supposition that it would be specially beneficial for the rush on account of its possession of Cl. What the writer

found out is that KCl is superior to K_2SO_4 in producing the rush of better quality, as the soil culture also proved.

(5) When $KMnO_4$ was used as the potassium source as well as a stimulant in so large a quantity as 25 kg/tan(K_2O), its beneficial effect on the crop could hardly be noticed. A considerable large number of tillers were, however, produced in the lots.

(6) Though $K_2Cr_2O_7$ is an oxidizing agent chemically resembling $KMnO_4$, it was found very harmful, making the crops wither in a short period. The negative ion (Cr_2O_7'') may be harmful when a valency of Cr is as high as 6. This crop in soil culture, however, did not suffer so much from the action of this salt as in sand culture. Further investigation will be necessary to determine its useful concentration.

(7) In the present experiment KBr and KI gave some interesting results; KBr-lot gave an yield of 94% as high as that of KCl-lot, but gave so bad a result that the plants all died. Further investigation is necessary as to the quantity of these salts useful as a stimulant.

(8) $KClO_3$ was harmful, but it was not so harmful as $K_2Cr_2O_7$ or KI. The harm it did to the rush was noted to be slack in appearing in comparison with that of $K_2Cr_2O_7$ or KI, and that some plants could withstand complete withering although the stems were slender and weak.

(9) The author's attention has also been directed toward the relative position on the periodic chart of the atoms of which negative parts of compounds are composed.